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Mycena cinereoalba sp. nov. from Qinghai province, China

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Abstract– From specimens collected in Sanjiangyuan (Source of Three Rivers) region, Qinghai Province, China, *Mycena cinereoalba* is described based on its morphological characteristics and a molecular phylogenetic analysis using the internal transcribed spacer (ITS) region and nuclear ribosomal RNA large subunit (nrLSU) sequences. The phylogenetic relationship inferred from the ITS and nrLSU sequence data matrices confirmed that this new species is different from other species of *Mycena* for which DNA sequences are available. The species are comprehensively described and illustrated in detail with photographs. Phylogenetic relationships among the species were analyzed, and two phylograms were provided in this study.

Key words–*Mycenaceae, Agaricales, Basidiomycota,* taxonomy

Introduction

Mycena (Pers.) Roussel is the largest genus in *Mycenaceae* (*Agaricales*, *Basidiomycota*). It is a widely distributed, resource-rich genus with more than five hundred known species in the world, most of which are saprophytic (Kirk & al. 2008). The main characteristics of this genus are: basidiomata are small; pileus is conical, parabolic or bell-shaped, thin and membranous, with or without stripes on the surface, and nearly smooth, and some species are hygrophanous; cap is thin; lamellae are adnate, decurrent or arcuate; stipe is

cylindrical, hollow, and fragile; spores are smooth and hyaline; the spore print is white; and cystidia are present and intricate. *Mycena* species are usually distributed in coniferous and other woody forests and are rarely found near grasses, mosses, and ferns (Pegler 1986; Singer 1986). Fungi have an important ecological, economic, and scientific research value (Boa 2004; Peters & Spiteller 2007); and in recent years, *Mycena* species have provided a new theoretical basis for the breeding and protection of orchids (Guo & al. 1997, 2019).

During an investigation of macrofungi in the main forested areas of the "Source of Three Rivers" region in Qinghai Province, China, an unidentified species was found at different locations in 2019–20. After further microscopic observation and phylogenetic analysis based on ITS and nrLSU sequences, it is described here as a new species.

Materials and methods

Specimens were collected and color photographs of the habitats of the fresh fruiting bodies were taken after rainy days. To ensure their integrity, ecological, habitat and morphological characteristics were recorded in the field. Some tissues were dried using silica gel in a sealed plastic bag, while others were dried in an electric oven and stored in a –20°C refrigerator at the Herbarium of Plant Pathology, Qinghai University, Xining, China (QHU).

Macromorphological descriptions of the basidiomata, including the size, shape, and surface characteristics of their pileus, lamellae, and stipe, were based on the fieldnotes and photographs. Color codes followed Kornerup & Wanscher (1978).

Micromorphological data were based on observations of the unarmed sections of the dried specimens, including the spores, basidia, pileipellis, and stipitipellis. Small pieces of dried specimens were revived in 5% KOH, stained with 1% Congo Red when necessary, and observed with an Olympus CX31 microscope. Twenty basidiospores and 10 basidia were measured in each collection. Size was indicated as "a–b × c–d". *Q* referred to the ratio of length to width in an individual spore, and *Qm* referred to the mean of the *Q* values.

Genomic DNA was extracted from the dried basidiocarps using the modified CTAB method (Doyle 1987). The primer pairs ITS4 and ITS5 and LR0R and LR7 (White & al. 1990; Vilgalys & Hester 1990) were used for PCR amplification of the ITS and nrLSU, respectively. PCR amplifications were performed with a total volume of 25 µL. The PCR procedure for the ITS was: predenaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing

at 55°C for 30 s, elongation at 72°C for 60 s; and a final extension at 72°C for 10 min. The PCR procedure for the nrLSU was: predenaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 40 s, elongation at 72°C for 1.5 min; and a final extension at 72°C for 10 min. Amplified PCR products were examined by gel electrophoresis using 1% agarose gel. Electrophoresis was conducted in a 1×TAE buffer for 30–40 min with a voltage of 120 V. Purification and sequencing were conducted by Lanzhou Tianqi Gene Biotechnology Co., Ltd. The primers used in the PCR were the same as those used in sequencing. The newly generated sequences have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Related GenBank sequences were identified by BLAST searches and retrieved for analysis. Alignments were constructed separately for gene fragments by the Multiple Alignment using Fast Fourier Transform (MAFFT) software version 7 (https://mafft.cbrc.jp/alignment/server) (Katoh & Standley 2013) and then manually adjusted in BioEdit (Hall 1999). The best base substitution model was obtained by ModelTest (Nei & Kumar 2000; Tamura & al. 2013). A phylogenetic tree was constructed in MEGA 5.2 using the Maximum Likelihood (ML) method with 1000 bootstrap replicates.

Taxonomy

Mycena cinereoalba L.C. Bai & Qiang, **sp. nov.** FIGS 1, 2

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Differs from *Mycena pura* by its grayish-white mature pileus, its gray lamellae, and its gray stipe with short fibrous white villi.

TYPE: China, Qinghai Province, Sanjiangyuan region, Jiangxi Forest Farm, 32.0754°N 97.0020°E, alt. 3501 m, 22 Aug 2020, L.C. Bai & T.T. Qiang (**Holotype**, QHU20461; GenBank OK091026, OK091029).

ETYMOLOGY: *cinereoalba* refers to the grayish white color of the basidiomata.

Basidiomata small. Pileus 28 mm diam, bell-shaped to hemispherical, nearly applanate when mature, grayish white, with stripes at the edge, hygrophanous, nearly smooth, and sticky when wet. The context is thin and white. Lamellae 3–5 mm broad, unequal, nearly closed, adnate, and nearly arcuate, gray and wrinkled on the surface, with a transverse vein between them, wavy at the edge. Stipe 35 × 6 mm, central, cylindrical, gray, with stripes and short, fibrous white villi on the surface; hollow, fragile and easily cracked, with a white basal mycelium. Odour, like carrots, though not distinctive.

Fig. 1. *Mycena cinereoalba* (holotype, QHU20461): basidiomata in the field.

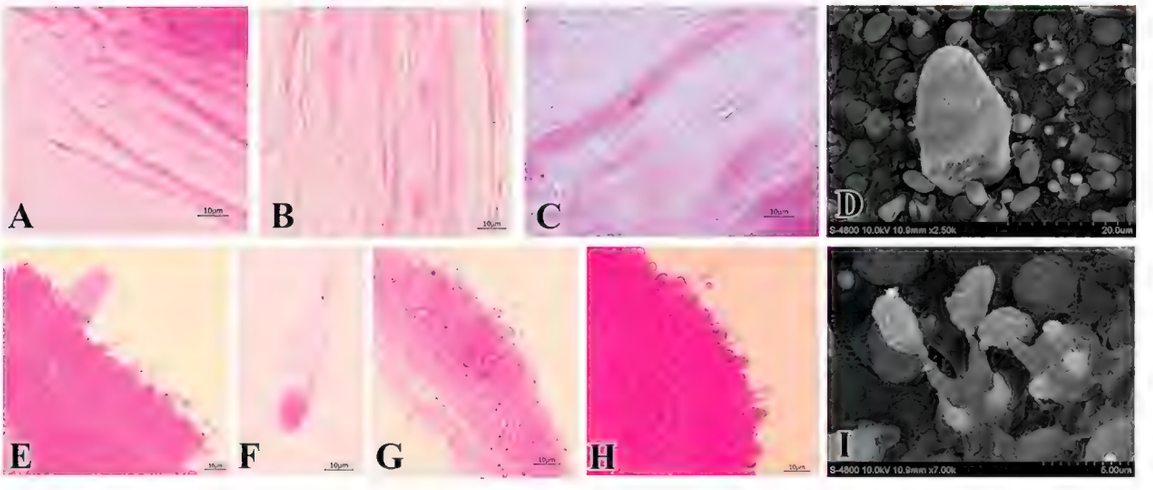

Fig. 2. *Mycena cinereoalba* (holotype, QHU20461): A. pileipellis; B. stipitipellis; C. stipititrama; D, F. cystidia; E. cystidia and basidia; G–I. basidia, stipititrama, and basidiospores. Scale bars = 10 μm.

Basidiospores 6.25–11.25 × 2.5–3.75 μm, Q = 1.67–3.0, Qm = 2.23. long ellipsoid or ellipsoid to cylindrical, amyloid or weakly amyloid, smooth, hyaline, thin-walled, some with oily contents. Basidia 25.0–32.5 × 5.0–7.5 μm, clavate, smooth, colorless, 2–4 spored. Cheilocystidia 52.5–65.0 × 12.5–18.75 μm, clavate and subcylindric. Stipititrama nearly parallel. Pileipellis 18.75–25.0 μm wide irregular. Stipitipellis 5.0-20.0 μm wide nearly parallel. Clamp connections presented on all hyphae.

Habit and habitat: Scattered or grouped in conifer forest.

Additional specimens examined: **CHINA, Qinghai Province, Sanjiangyuan region**, Dongzhong forest district, 32.5039°N 97.6011°E, alt. 3850 m, 18 Aug 2019, L.C. Bai & T.T. Qiang (Holotype, QHU19358).

Based on molecular information combined with distinctive morphological data, this is proposed as a new species of *Mycena* from China.

Phylogeny

ITS analysis

Fig. 3

The ITS dataset included 41 sequences from 18 *Mycena* species and one *Maramius siccus* outgroup. Through BLASTn analysis, the ITS sequence of the new species (OK091026, length 663 bp) was 89.49% similar to a Chinese collection of *Mycena pura* (MW855913), with a 99% query coverage. In the ITS phylogram, *Mycena cinereoalba* (OK091026) clustered in a branch with *M. pura* (MW855913), and was a sister species of *Mycena seminau* (KF537250, NR154170), *Mycena sinar* (KF537249, NR154169), and *Mycena sinar* var. *tangkaisinar* (KF537251, NR154171).

nrLSU analysis

Fig. 4

The nrLSU dataset included 32 sequences from 19 *Mycena* species and one *Maramius siccus* outgroup. The nrLSU sequence of the new species (OK091029, length 1050 bp) was 98.74% similar to a Chinese collection of *Mycena* cf. *pura* (LC164935), with a 97% query coverage. In the nrLSU phylogram, *M. cinereoalba* (OK091029) clustered in a branch with *Mycena cf. pura* (LC164935), and was a sister species of *Mycena rosea* (MK278406).

Discussion

Mycena cinereoalba is well characterized by its grayish-white pileus, with stripes at the edge, context is thin and white, its lamellae are gray and wrinkled on the surface, with a transverse vein between them. The phylogenetic tree inferred from the ITS sequences showed that *M. cinereoalba* clustered with *Mycena pura* (MW855913). The colors of *M. pura* are variable, the pileus is typically lilac to purple in color when young. The lamellae are attached to the stem by a tooth and are white or slightly pink to purple in color, developing

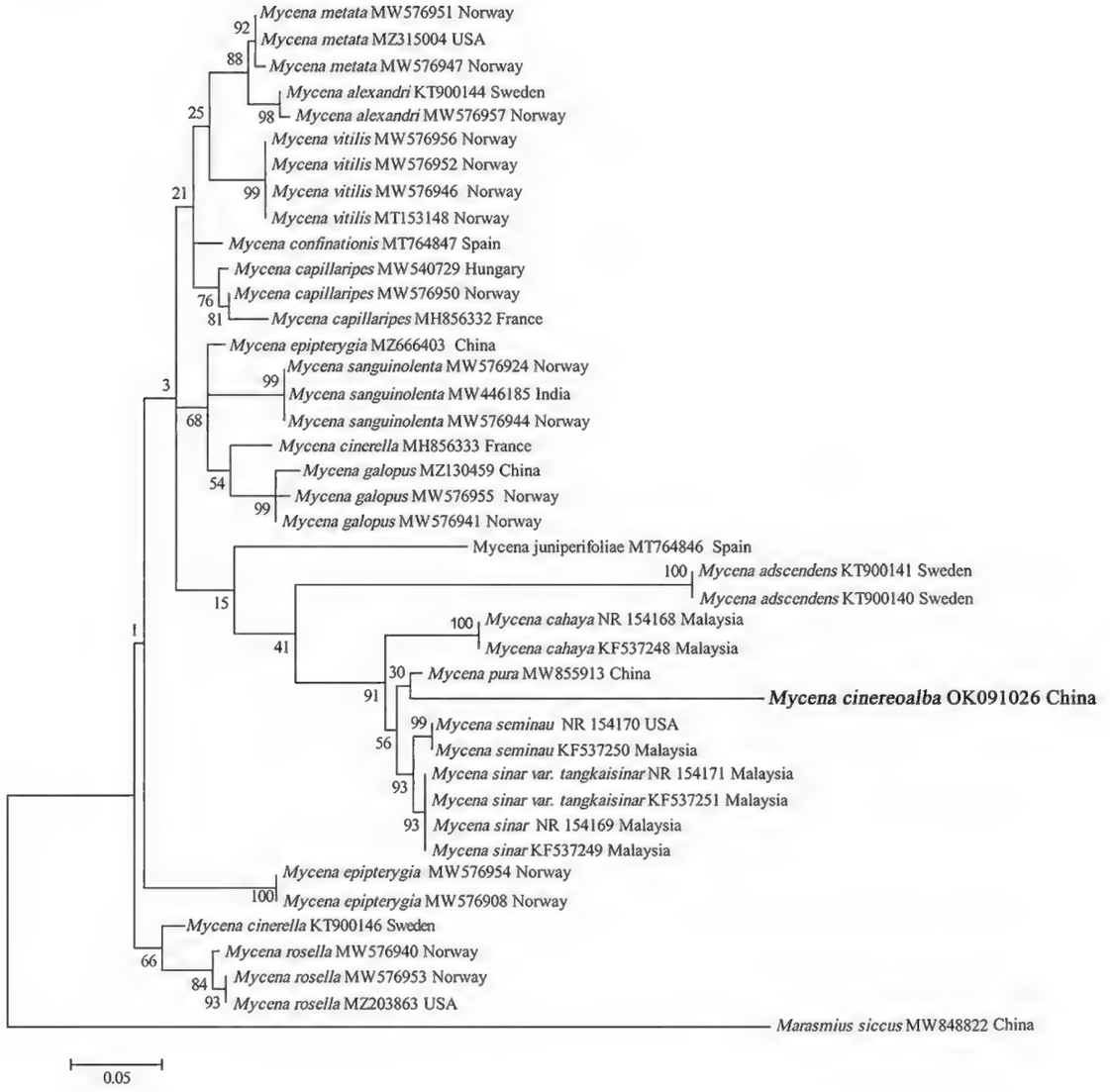
Mycena metata MW576951 Norway
Mycena metata MZ315004 USA
Mycena metata MW576947 Norway
Mycena alexandri KT900144 Sweden
Mycena alexandri MW576957 Norway
Mycena vitilis MW576956 Norway
Mycena vitilis MW576952 Norway
Mycena vitilis MW576946 Norway
Mycena vitilis MT153148 Norway
Mycena confinationis MT764847 Spain
Mycena capillaripes MW540729 Hungary
Mycena capillaripes MW576950 Norway
Mycena capillaripes MH856332 France
Mycena epipterygia MZ666403 China
Mycena sanguinolenta MW576924 Norway
Mycena sanguinolenta MW446185 India
Mycena sanguinolenta MW576944 Norway
Mycena cinerella MH856333 France
Mycena galopus MZ130459 China
Mycena galopus MW576955 Norway
Mycena galopus MW576941 Norway
Mycena juniperifoliae MT764846 Spain
Mycena adscendens KT900141 Sweden
Mycena adscendens KT900140 Sweden
Mycena cahaya NR 154168 Malaysia
Mycena cahaya KF537248 Malaysia
Mycena pura MW855913 China
Mycena cinereoalba OK091026 China
Mycena seminau NR 154170 USA
Mycena seminau KF537250 Malaysia
Mycena sinar var. tangkaisinar NR 154171 Malaysia
Mycena sinar var. tangkaisinar KF537251 Malaysia
Mycena sinar NR 154169 Malaysia
Mycena sinar KF537249 Malaysia
Mycena epipterygia MW576954 Norway
Mycena epipterygia MW576908 Norway
Mycena cinerella KT900146 Sweden
Mycena rosella MW576940 Norway
Mycena rosella MW576953 Norway
Mycena rosella MZ203863 USA
Marasmius siccus MW848822 China
0.05

FIG. 3. Phylogenetic relationships of *Mycena* species inferred from ITS sequences using Maximum Likelihood, with *Marasmius siccus* as the outgroup. Bootstrap support is indicated at nodes. The newly described species is indicated in bold.

cross-veins when matured. The stipe is smooth (or has tiny hairs) on the surface. It tastes radish and growing on forest debris under hardwoods and conifers, growing in spring, summer and fall. The spores are long-elliptical or nearly cylindrical. The cheilocystidia and pleurocystidia are scattered or abundant, and fusoid-ventricose to widely fusiform (Kibby 2017).

The phylogenetic tree inferred from the nrLSU sequences showed that *M. cinereoalba* clustered with that of *Mycena* cf. *pura* (LC164935). The microscopic characteristics of *Mycena* cf. *pura* and *M. pura* are similar (as implied by their names). *Mycena cinereoalba* is a sister species to *M. rosea*. The pileus of *M. rosea* has a light pink to pink hue, and its edge has short stripes. It smells strongly

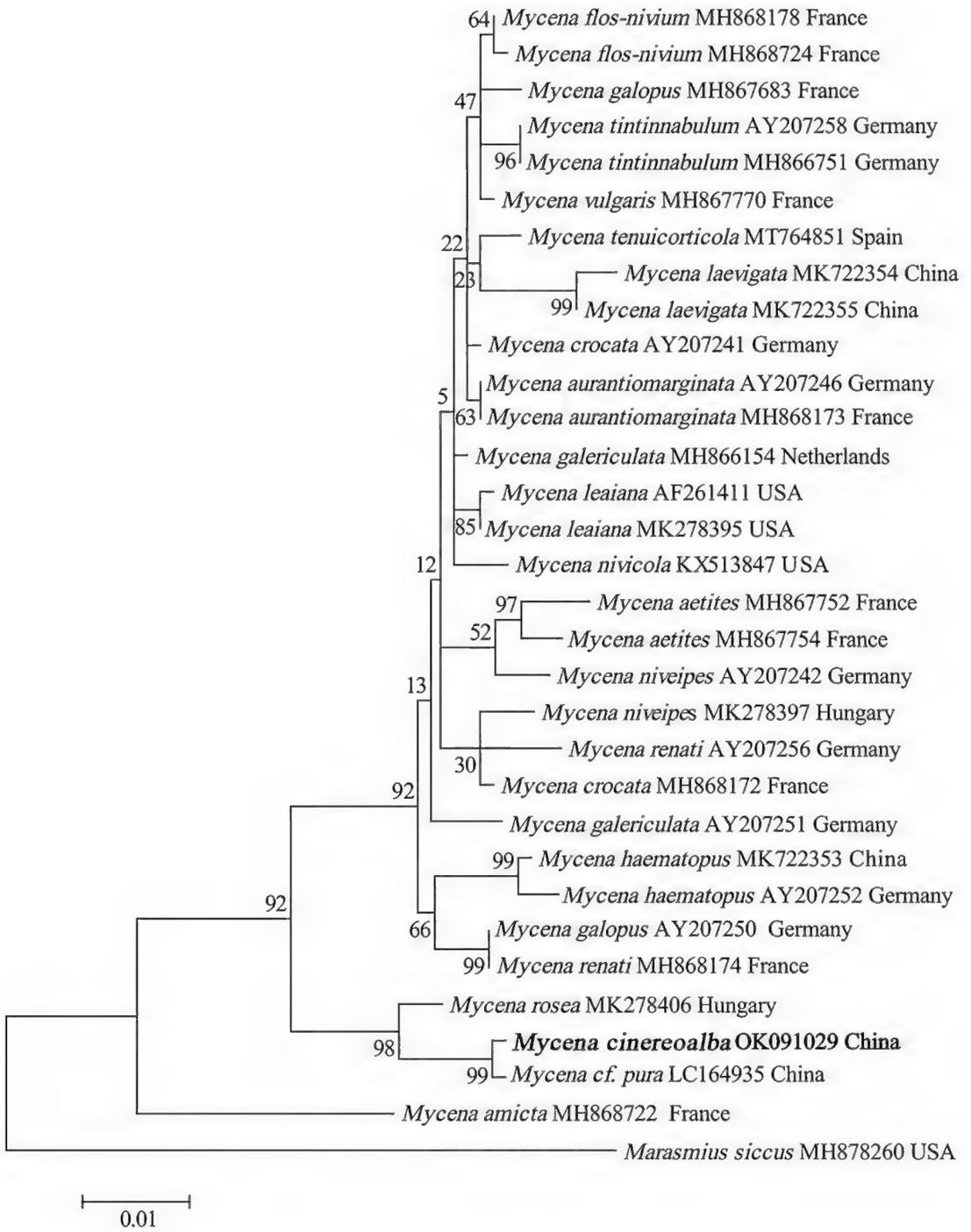
64 Mycena flos-nivium MH868178 France
Mycena flos-nivium MH868724 France
47 Mycena galopus MH867683 France
Mycena tintinnabulum AY207258 Germany
96 Mycena tintinnabulum MH866751 Germany
Mycena vulgaris MH867770 France
22 Mycena tenuicorticola MT764851 Spain
23 Mycena laevigata MK722354 China
99 Mycena laevigata MK722355 China
Mycena crocata AY207241 Germany
Mycena aurantiomarginata AY207246 Germany
5 63 Mycena aurantiomarginata MH868173 France
Mycena galericulata MH866154 Netherlands
Mycena leaiana AF261411 USA
85 Mycena leaiana MK278395 USA
12 Mycena nivicola KX513847 USA
97 Mycena aetites MH867752 France
52 Mycena aetites MH867754 France
Mycena niveipes AY207242 Germany
13 Mycena niveipes MK278397 Hungary
Mycena renati AY207256 Germany
30 Mycena crocata MH868172 France
92 Mycena galericulata AY207251 Germany
99 Mycena haematopus MK722353 China
Mycena haematopus AY207252 Germany
92 66 Mycena galopus AY207250 Germany
99 Mycena renati MH868174 France
Mycena rosea MK278406 Hungary
98 Mycena cinereoalba OK091029 China
99 Mycena cf. pura LC164935 China
Mycena amicta MH868722 France
Marasmius siccus MH878260 USA
0.01

Fig. 4. Phylogenetic relationships of *Mycena* species inferred from nrLSU sequences using Maximum Likelihood, with *Marasmius siccus* as the outgroup. Bootstrap support is indicated at nodes. The newly described species is indicated in bold.

raphanoid. The base of its stipe is normally clavate. Its spores are amyloid, cheilocystidia and pleurocystidia were present, and clamp connections were observed. It is widely reported in Britain, especially in the southern counties of England.

By investigating and combining the diversity of macrofungi in the Jiangxi Forest Farm in Qinghai Province, this study provides important theoretical support for the study of *Mycena* in China and is beneficial to the conservation and utilization of macrofungal germplasm resources.

Acknowledgments

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